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Clinical and Scientific Aspects of Botulinum A Toxin

Gary E. Borodic, MD, L. Bruce Pearce, PhD†, Eric Johnson, PhD‡, and Edward Schantz, PhD§*

During the past decade, injectable botulinum toxin has been used in ophthalmic and neurologic clinical practice as an unusual and effective form of therapy for involuntary movements and imbalances of muscle tone. This scientific and clinical technology, however, still has significant imperfections, and further development in basic and clinical science is needed. Biologic activity standardization of the toxin using the mouse LD 50 assay during the manufacturing process has been problematic with respect to reproducibility, issues of toxin antigenicity after repeated injections remain unclear, and the most suitable injection methods are as yet undetermined. Such inconsistency can have significant implications on medicinal effectiveness and safety. Because of the pharmacologic importance of the preparation process, the first part of this article reviews the basic principles in drug preparation. Subsequent sections discuss ophthalmic and neurologic clinical studies. Critical review and understanding of toxin preparation can give physicians a basis for critical evaluation of the drug preparation being used clinically.

BASIC SCIENCE OF BOTULINUM TOXIN

Botulinum neurotoxins are produced by certain strains of the bacterial species *Clostridium botulinum* and *Clostridium baratii*.[38] The toxins are classified into seven serotypes, A through G. The botulinum neurotoxins comprise a family of pharmacologically similar poisons that block acetylcholine release from peripheral nerves and cause a flaccid paralysis. Type A botulinum toxin is the serotype currently used in clinical practice.

Toxin Purification and Properties

History of Toxin Purification. The first recorded attempts to purify the type A toxin from culture were made in 1928 by Snipe and Sommer[79] at the Hooper Foundation at the University of California. They showed that 90% to 95% of the toxin could be precipitated from a deep broth culture of *C. botulinum* type A by the addition of acid to lower the pH to 3.5. About 20 years later, Lamanna et al[50], starting with the precipitated toxin, obtained the type A toxin in crystalline form, and Duff et al[24] improved the method that is the basis for the procedures now used to purify toxin for use in the treatment of humans. Lamanna et al[51] discovered that the purified toxin could be separated into nontoxic and toxic components when they found that a nontoxic component precipitated red blood cells, leaving the toxin in solution. Putnam et al[60] showed that the crystalline toxin moved as a single sub-

* Clinical Instructor, Massachusetts Eye and Ear Infirmary, Harvard Medical School; and Boston University School of Medicine, Boston, Massachusetts
† Assistant Professor, Boston University School of Medicine, Boston, Massachusetts
‡ Associate Professor, University of Wisconsin Food Research Institute, Madison, Wisconsin
§ Emeritus Professor, University of Wisconsin Food Research Institute, Madison, Wisconsin

stance in electrophoresis, with a molecular weight of 900,000. Wagman and Bateman[89] also showed that the toxin moved in the ultracentrifuge as a single substance with a sedimentation coefficient of 19S and a molecular weight of 900,000 at pH 5.6, but at pH 7.3, the toxin component (neurotoxin) dissociated and moved as a much smaller molecule (7S). Later DasGupta and Boroff[17] showed that the neurotoxin could be separated from the nontoxic proteins by column chromatography.

Properties of Crystalline Toxin and Neurotoxin. The crystalline type A toxin contains 16.2% nitrogen and, thus far, has been found to be composed of only biologically active amino acids[13,80] for both the neurotoxin and the nontoxic proteins. The isoelectric point of the crystalline type A toxin is pH 5.6. Under slightly acidic conditions, pH 3.5 to 6.8, the neurotoxic component of 150,000 Mr (molecular weight) is bound noncovalently to the nontoxic proteins in such a manner as to preserve or help stabilize the secondary and tertiary structure upon which toxicity is dependent. Under slightly alkaline conditions (pH greater than 7.1) and in the blood and tissues of animals and humans, the neurotoxin is released from the toxin complex. The primary structure[3,85] of the neurotoxin is such that the resulting shape (secondary and tertiary structures) causes highly specific binding and block of acetylcholine release at the myoneural junction.

All of the neurotoxins are synthesized as intact protein molecules with a molecular weight of about 150,000 with low toxicity and are released from the bacterium during culture.[82] Those from proteolytic (Group I) *C. botulinum* strains are cleaved by extracellular proteases produced by the bacterium into dichain molecules consisting of a heavy (H) subunit of about 100,000 MR and a light (L) subunit of about 50,000 Mr. The two chains are covalently linked by a disulfide bond and by noncovalent bonds. During cleavage the molecules undergo a molecular change that increases toxicity.[19] Nonproteolytic (Group II) *C. botulinum* strains do not have the endogenous nicking protease, and their toxins are isolated from the culture as single-chain 150,000 Mr molecules. Neurotoxins have been purified for all serotypes except for type G. The H and L chains of the neurotoxin can be separated after reduction by chromatography. The isolated chains are not toxic by themselves but can be recombined under carefully controlled conditions to active toxin.[45,54,83] All of the neurotoxins have high specific toxicities ranging from 1×10^7 to 1×10^8 mouse LD50 per milligram of protein.[82]

Little is known about the nontoxic proteins associated with the neurotoxin except that at least one has hemagglutinating properties. However, all apparently have a role in the stability of the neurotoxin because the type A toxin of high 19S sedimentation rate and 900,000 molecular weight is most stable to digestive and metabolic enzymes.[63,64] The nontoxic proteins may also help to stabilize toxicity during the injection of toxin into human tissues, but no data are available that directly compare the efficacy of crystalline toxin and isolated neurotoxin. Unless a suitable means of stabilizing the neurotoxin can be achieved, it is apparent that the crystalline toxin (neurotoxin bound to nontoxic proteins) must be the choice for treatment in humans.

Because the toxic properties of the crystalline toxin are due to its structural shape, it is readily detoxified by temperatures above 40°C and on high dilution (milligrams to nanograms), which destroys the shape, particularly at pHs above 7. The loss caused by dilution can be prevented by performing dilution with solutions containing a small amount of albumin or gelatin and by avoiding bubbles, which can lead to stretching and surface denaturation.[66]

Production and Purification of Type A Toxin for Treatment in Humans. The production of type A toxin by injection into muscle nervous tissue necessitates that the production in culture and the purification be performed so that the toxin is not exposed to substances that might be carried through the process in trace amounts and cause undue reactions in the patient. Johnson et al[43] have described a process in which the toxin production is carried out in culture medium composed of 2% hydrolyzed casein, 1% yeast extract, and 0.5% dextrose in 12-L carboys. The culture medium contains no animal meat products of any kind, which could present antigenic hazards if slight amounts were carried through toxin preparation. Toxin production is done in this medium with a high toxin producing strain of *C. botulinum* and allowed to ferment at 37°C for 3 to 4 days or until the culture has attained maximum growth and the cells have lysed completely and liberated the toxin into the spent culture.

The toxin is removed from the spent culture for further purification by precipitation with

acid, as indicated previously,[79] forming a mud-like material about 1/100 of the culture volume containing the toxin. This mud-like precipitate is washed with water and the toxin extracted with salt solution weakly buffered with phosphate at pH 6.5 to 6.8. After another precipitation with acid, the toxin is precipitated with ethanol at −5°C, similar to procedures used for the precipitation of certain blood proteins. The final step in the purification is crystallization of the toxin dissolved in a phosphate-buffered solution at pH 6.8 with the addition of enough 4 M ammonium sulfate to bring the concentration to 0.9 M. Crystallization takes place within a few days at 4°C. During this process, the toxin is not exposed to any enzymes added to assist in the purification or to columns of synthetic resins or dangerous organic solvents of any kind. The toxin will remain stable and active for many years suspended in the ammonium sulfate solution.

The crystalline toxin, when dissolved in 0.05 M sodium phosphate buffer at pH 6.8, has a maximum ultraviolet absorption at 278 nm. The amount of absorption at 278 nm divided by 1.65, the extinction for 1 mg of toxin,[80] yields the total milligram of toxin in a particular sample and in the entire preparation. From this value the specific toxicity of the toxin per milligram is obtained by determining the LD50 from the serial dilutions of a sample of the toxin that will kill 50% of a group of white mice.[68] A good and acceptable preparation of the crystalline type A toxin must have a specific toxicity of 3×10^7 mouse LD50 per mg. Another important measurement on a preparation of the toxin used for human treatment is the absorption at 260 nm, which is a measure of the nucleic acid material carried over in the purification. The 260/278 nm ratio is used as one measure or index of purity of the toxin. This ratio for the most highly purified type A toxin has been determined to be close to 0.5, but the value is difficult to attain even under the most careful purification and repeated crystallization of the toxin. Preparations in our laboratory attain a ratio of 0.55 or slightly less and are considered highly purified and should be good for human treatment. Even at a value of 0.6, the amount of nucleic acid-absorbing material would only be 0.08 to 0.1%. This ratio, along with gel electrophoresis analyses, defines the purity of the type A toxin used for human treatment.

For human treatment, the crystalline toxin must be diluted from milligram concentrations to nanogram concentrations. To stabilize the toxin on such great dilution, small amounts of another protein such as gelatin or albumin are added to the saline solution used for dilution. This diluted toxin solution is filtered for sterility and dried by lyophilization in small vials for dispensing. Although drying helps preserve sterility in case of contamination, it also causes the inactivation of some of the toxin. A final mouse assay must be done for dosage purposes and to meet FDA requirements.

Method of Assay. Because the immunologic properties of the neurotoxin are independent of its toxic properties, the only means to evaluate the effectiveness of the toxin for treatment in humans is by an animal assay for toxicity.[67] The standardized mouse assay[68] carried out with the Food and Drug Administration (FDA) reference standard should be used. Assays based on immunologic properties of the toxin yield active as well as inactive toxin and are not recommended for the toxin used in the treatment of humans.

Toxicity in Humans

Botulinum toxin is used clinically by injection of 1 to 300 mouse units depending on the condition being treated. Obviously, it is important that the dose injected be sufficiently low to prevent intoxication. Patients with accidental botulism from toxin-contaminated food have shown symptoms of botulism and, occasionally, have died with 0.1 to 1 μg (100–1000 ng or 3000–30,000 mouse LD50 [MLD50 or U].[56,57] Scott and Suzuki[75] determined that the intramuscular LD50 for juvenile monkeys *(Macaca fascicularis)* was approximately 39 mouse U/kg (approximately 1.25 ng/kg) body weight. Herrero et al[39] reported a similar lethal dose of 40 U/kg by intravenous injection in *Macaca rhesus*. No data on the intravenous toxicity for humans are available for these types, but humans are probably at least as sensitive as guinea pigs and expectedly have similar sensitivities as monkeys.

Immunology of Botulinum Toxins

An important factor in the use of botulinum toxin as an injectable protein drug is the elici-

tation of antibodies and possibly other immunities in treated individuals. The minimum dose of toxoid to elicit immunity in humans varies with the individual and toxoid preparation[1,36] but is probably similar to the immunologic response to tetanus toxoid.[36] The amount of toxin needed to elicit antibodies is thought to be greater than the lethal dose for humans. Therefore, antibodies should not develop with current maximum treatment schedules (less than 300 U per treatment). However, if toxin is mishandled during its formulation and drying or during incorrect rehydration by physicians, inactive toxin (toxoid) will be formed, which could promote antibody formation.

Botulinum toxin and antibodies against botulinum toxin can be detected in sera from humans by in vitro assays using specific antitoxins. One International Unit (IU) of antitoxin will neutralize 10,000 mouse LD50 except for type E, for which 1 IU will neutralize 1000 LD50. The method currently being developed is the amplified enzyme-linked immunosorbent assay (ELISA) (WH Lee, 1990, personal communication).[53] By this method it is possible to measure 10 picograms or less of protein, including botulinum toxin and antibodies to botulinum toxin in human serum. The ELISA method is a linear and quantitative method with a sensitivity comparable to that of the mouse bioassay but differs from the mouse test in that it will detect some but not all forms of biologically inactive but ELISA-active botulinum neurotoxin. The ELISA also can be used to detect hemagglutinin A of the toxin complex. The major limitation in the amplified assay is the quality and behavior of the antibodies. As botulinum toxin therapy becomes more widely used, it may be necessary to have available highly specific and uniform primary and secondary antibodies and possibly Fab′ or F(ab)′2-labeled reagents as second antibodies to lower background color formation.

Use of Other Types of Botulinum Toxin

Seven primary serotypes of botulinum toxin (A–G) are recognized experimentally by the ability of polyclonal antibodies raised against one type to neutralize toxicity in the mouse assay. In patients in whom immunity to botulinum toxin type A develops, it may be necessary to use other serotypes of botulinum toxin, particularly types that do not immunologically cross-react. Cross-reactions have been detected between toxin types C and D[14] and between types E and F.[15,21] However, larger quantities of antitoxin for one of the types may be required to neutralize much smaller doses of the other.[91] When prepared against pure toxoided neurotoxin, antisera generally contain a higher titer of antibodies that react with the heavy chain compared with the light chain.[46,47]

The nontoxin components of some types of toxin complexes have been reported to be antigenically similar to nontoxic proteins of other serotypes.[47,81] Antibodies obtained from a hemagglutinin fraction isolated from type A toxin complex reacted with nontoxic protein fraction of type B.[19] Nontoxic proteins of types C1 and D,[82] A and F, and E and F[64,65] also were reported to be antigenically related or identical.

Evidence has been found that various serotypes of botulinum neurotoxins bind to different "receptors" and with different avidity on nerve termini. Competition binding studies in vitro have indicated that different receptors are involved in binding types A and B,[2,45,91] D,[58] and F.[88]

CLINICAL PHARMACOLOGY

Botulinum A toxin has demonstrated unique pharmacologic properties as a neuromuscular blocking agent. When injected into striated muscle, the toxin irreversibly blocks the release of acetylcholine and can sustain partial denervation for 3 to 5 months.[6,7,26,29,72] Observed clinical effects from partial denervation include a reduction of the contractile force of the muscle, a decrease in muscle mass from fiber atrophy, and the reduction of spasm frequency and resting muscular tone. The degree of this effect is dependent on the dose injected, i.e., more atrophy, weakness, and tone reduction result from higher doses. The pharmacologic effect occurs by a three-step process beginning with the binding of a high molecular weight subunit of the toxin to receptors localized at the nerve terminals followed by endocytotic internalization of a low molecular weight subunit of the toxin.[44,76,77,78] Once internalized, a low molecular weight subunit is responsible for the blockade of exocytotic release of neurotransmitter by a mechanism that remains elusive.[44,76,77,78] Blockage of neurotransmitter

release begins to occur within minutes to hours. After 2 to 3 weeks, an extrajunctional spread of acetylcholine receptors and acetylcholinesterase at the postsynaptic membrane is observed and indicates denervation.[9,10,22,23] Also, within several weeks, collateral axonal sprouts develop from preterminal motor axons followed by the establishment of functioning neuromuscular junctions.[22,23] Apparently, not all collateral axonal sprouts establish neuromuscular junction. Some sprouts lead to "dead ends."[22,23] Within 3 to 4 weeks, atrophy of muscle fibers can be noted in a pattern similar to that in nerve transections. Figure 1 demonstrates the large degree of fiber size variability 6 weeks after botulinum toxin injection as compared with after saline controlled injections. Collateral sprouting and spreading of acetylcholinesterase has been demonstrated in muscles in humans 5 to 6 weeks after injection with the toxin.[9,10] After 10 to 12 weeks, evidence of denervation recedes as muscle fiber size variability diminishes and extrajunctional acetylcholinesterase staining decreases.[10,84] Biopsy specimens of human muscle taken at greater than 6-month intervals after the last botulinum A toxin injection often show no evidence of abnormalities in acetylcholinesterase staining pattern or muscle fiber size variability.[9,10] The spread of the toxin from the point of injection produces a gradient of denervation within a specific geometric field.[11] This spread is not necessarily confined by skeletal or fascial planes because histologic evidence of denervation has been demonstrated in muscles contiguous to those injected.[11]

Although botulinum A toxin can cause substantial denervation, there has been no long-term adverse effect on muscle tissue after repeated injections.[9,11] Fibrosis, contracture, or permanent denervation has not been observed in histologic studies of orbicularis oculi muscle biopsy specimens taken during ptosis surgery from patients who have received repeated injections.

Resistance to the Toxin with Repeated Injections

Because injections must be repeated in most diseases for which the toxin is applied, the possibility of active immunization to the drug is a potential limiting factor. Antibodies have been demonstrated in 7 of 79 patients treated for spasmodic torticollis who have received frequent injections at higher doses (C Hathaway, 1991, personal communication).[70] Antibodies were not found in a small number of patients treated for blepharospasm for a relatively short period of time.[37] Sensitization, however, has occurred in small dose applications with repeated injections, such as for spasmodic dysphonia and occupational hand dystonias (C Hathaway, personal communication). The incidence of antibody formation in long-term therapy for blepharospasm or other applications is still unknown.

Figure 1. Denervation effects on striated muscle. Note the large degree of variability of muscle fiber size.

The long-established bioassay for antibodies to botulinum toxin (mouse assay) is identical to that used in the evaluation of botulinum toxoid effectiveness. Patient serum is mixed with a standardized test dose of botulinum toxin. The test dose is determined against a given quantity of antitoxin (C Hathaway, personal communication). If neutralizing antibodies are present within the patient serum mixed with a botulinum test dose, the mice will survive the lethal injection of a predetermined toxin quantity.

The presence of neutralizing antibodies within patient sera indicates active resistance and has been associated with the loss of effectiveness of repeated toxin injections (E Johnson, personal communication).

INDICATIONS

Strabismus

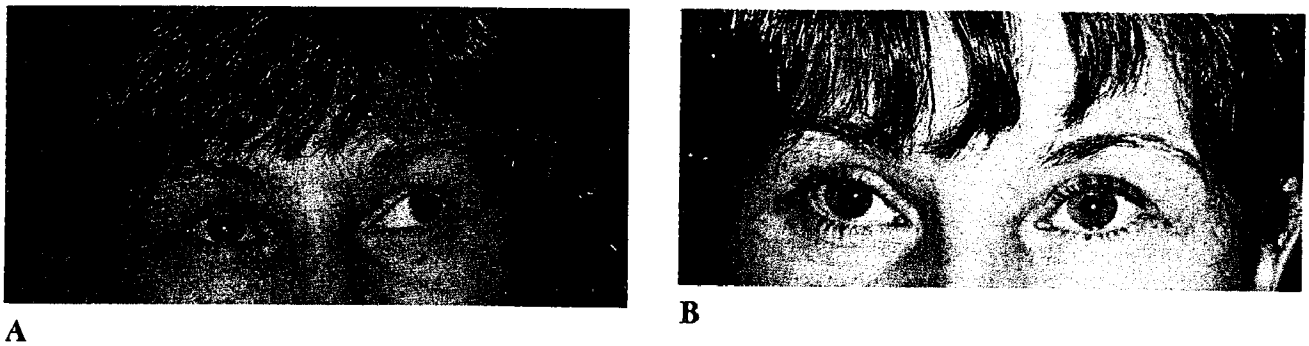
Strabismus was the first syndrome for which botulinum A toxin was extensively studied. When injected into recti muscles, a temporary denervation results in muscle weakening followed by an alteration of the position of the globe.[71,74] For example, the patient shown in Figure 2 had undergone four previous operations for strabismus yet remained 45 to 55 prism diopters exotropic. After 5 IU of botulinum A toxin was injected into the left lateral rectus muscle, the deviation was reduced to 15 prism diopters after 2 weeks. This patient demonstrated a substantial short-term benefit of the toxin injection. The long-term result of therapy appears to relate to the patient's fusion potential. The presence of relatively high fusion capability appears to stabilize and sustain alignment and is important to the ultimate success of this procedure and other surgical procedures. In children, an average of 68% improvement in esotropic deviation and 50% in exodeviation was noted based on a study of 356 patients.[71,74] In adults, a 65% improvement was noted in esotropia and a 61% improvement in deviation with exotropia based on data accumulated by observing 677 patients for an average of 17 months.[71,74] Repeated injections are common in all categories (45%).[71,74]

Toxin injections have been used for the treatment of acute and chronic sixth nerve palsies.[30,49,55,62,73] Although there has been clear benefit as measured by the improvement in horizontal deviation, the effect is generally not sustained unless there is active regeneration of the sixth cranial nerve. Transient medial rectus weakness is temporary and recedes as medial rectus reinnervation occurs. The procedure, however, has been thought to improve the eventual ocular motility by reducing the contracture formation within the antagonistic medial rectus muscle that may occur over a several month period during the early course of sixth nerve palsies. Treatment with botulinum toxin may reduce the number of patients needing vertical rectus muscle transposition surgery,[30,49,55,62,73] perhaps by reducing this medial rectus contracture. In chronic sixth nerve palsy, the administration of toxin may be helpful in increasing the efficacy of conventional surgery on horizontal deviations.[62]

The disadvantages of the application of botulinum toxin for strabismus include the common need for more than one injection and the findings that the alignment is probably not as stable as that achieved with conventional surgery. Excess denervation can result in a transient paralytic strabismus causing diplopia, and vertical deviation and transient ptosis are not uncommon (15% to 20%), and disappointing results have been reported in patients with paralytic and restrictive forms of strabismus.[71,74]

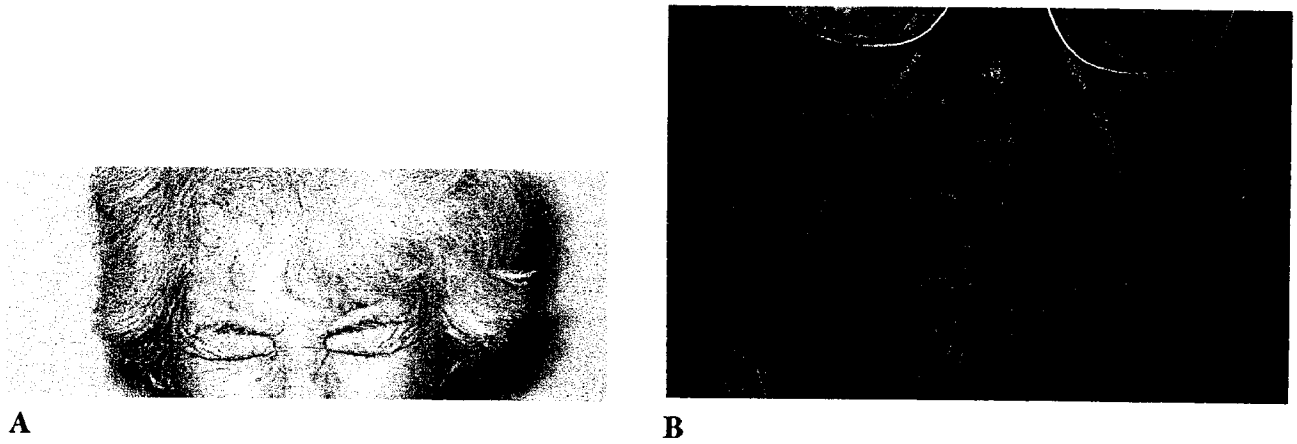
In summary, it is unlikely that botulinum A toxin will replace conventional surgical procedures for strabismus as the primary form of therapy in the future. However, the application of toxin can be viewed as a helpful adjunct to existing treatments.

Technique. The toxin injection procedure requires the use of electromyographic signal generated from a Teflon coated injection needle. This technique ensures that the needle is clearly within the appropriate muscle tissue. The needle is placed through the anesthetized conjunctiva, and the patient is asked to look to the right and left. As the electromyograph emits the signal as muscle tissue is impaled, the surgeon is assured that the needle is correctly positioned within muscle tissue. This procedure necessitates some knowledge and understanding of simple electromyographic equipment. Because globe perforations have occurred when this technique has been used, informed consent should be obtained. The potential for perforation and the relationship between technique and treatment efficacy underscore the need for training.



A B

Figure 2. *A* and *B*, Treatment of severe exotropia with lateral rectus injection of botulinum A toxin. A=Before injection; B=After injection.



A B

Figure 3. *A* and *B*, Involuntary blepharospasm with other involuntary facial movement defines Meige disease.

Figure 5. Hemifacial spasm with involuntary synchronous contractions of the muscles innervated by one facial nerve.

Figure 6. Adult-onset spasmodic torticollis with involuntary contractions of muscles rotating and tilting the head and flexing the neck.

Essential Blepharospasm and Meige Syndrome

Involuntary blepharospasm is defined as uncontrolled eyelid closure in the absence of primary ocular disease, such as keratitis or uveitis. The increased blinking or episodic spasmodic contractions of the eyelids usually compromise vision. Frequently, patients seek medical attention when driving or maintaining gainful employment becomes difficult. Involuntary movements are not just limited to the eyelids but may involve other muscles of the head and neck. This condition is termed *Meige syndrome*.[6,7,26,29,72] Meige syndrome is characterized by involuntary blinking (essential blepharospasm) plus involuntary facial grimacing, frowning, facial contortions, head titubations, spasmodic speech (spastic dysphonia), and various forms of spasmodic torticollis (Fig. 3*A* and *B*). This syndrome usually begins with blepharospasm as the presenting sign and progresses to other involuntary movements of the head and neck region in subsequent years. The age of onset is frequently in the fourth and fifth decade, and women are generally more commonly afflicted (60/40). Patients with Meige syndrome have a higher incidence of other family members with facial dyskinesia, bruxism, torticollis, and essential tremor of the head and hand. The condition has been described in identical twins.[5] Prior therapy for this condition has included the use of neuroleptic drugs and various forms of facial neurectomy and myectomy surgery.[16,35] Neuroleptic medication often lacks sustained efficacy[39] and occasionally results in serious systemic complications, including psychosis, urinary retention, lethargy, and gastrointestinal disturbances.

Although helpful, myectomy surgery is associated with both surgical risks of hemorrhage and potential facial disfigurement. Myectomy surgery also does not completely relieve symptoms of involuntary lid closure and often must be repeated.[16,35] Facial neurectomy has also been associated with disfigurement and the lack of efficacy, and the need for repeated procedures is not uncommon.[32,61]

Injections of botulinum A toxin into orbicularis oculi muscle have proved to be effective in controlling various forms of blepharospasm and facial spastic disease.[6,7,26,29,72] The toxin is injected into 4 to 6 points along the orbicularis oculi muscle and results in partial denervation over a 7- to 14-day period. The beneficial effect is generally maintained for a period of 10 to 14 weeks.[6,7,26,29,72] Toxin denervation results in a reduction in both the frequency of blinking and intensity of spasmodic contractions. Repeated treatments are almost always necessary to maintain these therapeutic actions. Because denervation of the injected muscle is partial, the patient retains a blink reflex that maintains a precorneal tear film and protects against exposure.

The injection points are outlined in Figure 4. Although these points have been derived empirically based on clinical observation, botulinum toxin injection points appear to have bearing on the response and complication rate. Frueh et al[33] have noted that lower lid injection, when placed medially, causes a higher incidence of diplopia. Presumably, the lower lid toxin injection can diffuse to the anteriorally positioned inferior oblique muscle to induce paralytic strabismus. The upper lid injection also should be positioned close to the lash line along the medial and lateral extent of the upper lid. These upper lid injection positions afford the greatest distance between the orbicularis oculi muscle injections and the muscular portion of the antagonist levator palpebrae superioris muscle. Toxin diffusion into the muscular portions of the levator muscle from upper lid injections can result in transient ptosis. Toxin-induced ptosis may persist several weeks to several months after injection. Multiple injection points over orbicularis muscle have been compared to single motor point injections.[8]

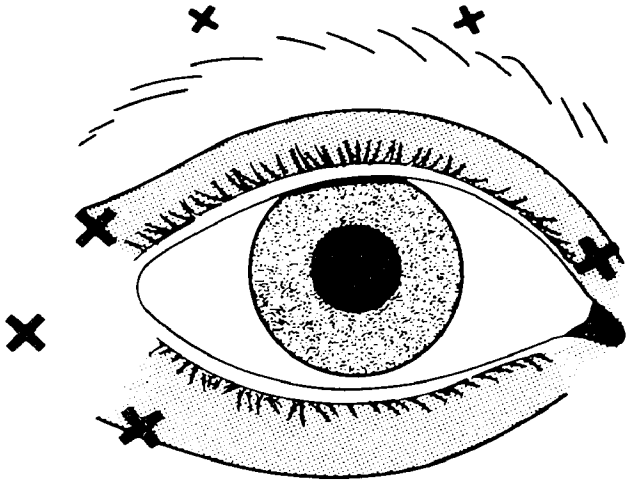


Figure 4. Usual injection points (x) for botulinum A toxin used to treat Meige syndrome.

From clinical observation, it appears the single motor point injections are inferior to multiple injections throughout this muscle. The reason may be that the innervation zone (distribution of neuromuscular junctions) is diffuse for this muscle.[8] Selective chemodenervation of the orbicularis oculi is the goal in the treatment of blepharospasm, and factors such as anatomic location and the injection of multiple points appear to be important factors in achieving this goal.

To date, patients have been treated with repetitive injections of botulism toxin for up to 7 years. Analysis of specimens taken during eyelid surgery for myectomy or ptosis does not provide evidence of long-term abnormalities in muscle fiber histology, provided that injections are not administered for 4 to 5 months before a muscle biopsy.[10] Furthermore, no adverse long-term effects have been demonstrated in clinical studies.[27]

The toxin also has been used successfully in conjunction with myectomy surgery for reducing the degree of surgery necessary to sustain beneficial effects.[25]

In addition to diplopia and ptosis, complications have included lid malpositions (<2%), lagophthalmos with exposure (<5%), and lid hematoma from injection.

Hemifacial Spasm

Hemifacial spasm is characterized by involuntary synchronous movement of muscles supplied by a single facial nerve. The observed involuntary eyelid closure is associated with other facial muscle contractions drawing the nasolabial fold superiorly, everting the lateral lower lip (Fig. 5). Patients often find this condition extremely disfiguring and functionally incapacitating. Normal facial expressions used in daily communication become interrupted by these involuntary movements. Unlike Meige syndrome, hemifacial spasm is a unilateral form of facial dyskinesia, with bilateral cases being very rare. These patients will often demonstrate weakness on the side in which the involuntary movements are occurring. Electromyographic studies have indicated denervation within the involved facial muscles of these patients.[20] A demyelination theory has been proposed to explain the cause of this condition. Based on electromyographic data,[59] it is thought that demyelination occurring within the intracranial portion of the seventh cranial nerve results in an ectopic site of excitation that propagates in an antegrade fashion with ephaptic transmission. This theory is consistent with the clinical findings of facial nerve weakness and synchronous involuntary contractions seen in these patients. Tortuous vessels at the base of the brain pressing on the facial nerve are implicated as a cause of this condition and represent a surgically correctable lesion.[42]

Treatment with neuroleptic medications has been totally ineffective. Jennetta et al[42] have proposed the use of posterior craniotomy with decompression of tortuous vessels away from the intracranial portion of the seventh nerve. Although the neurosurgical procedure has proved effective in some instances, patient and physician acceptance of this procedure is poor because the potential complications from neurosurgery are commonly considered unacceptable.

Botulinum A toxin has proved effective in relieving spasms in more than 95% of patients studied.[6,7] The toxin is injected into the usual points along the orbicularis oculi muscle, as well as into an additional point over the superior portion of the zygomaticus major and minor muscles. The total dose, however, tends to be less than that required for essential blepharospasm, probably because this condition is already associated with denervation. Generally, 10 to 20 IU represents a reasonable starting dose. The average duration of action is 5.1 months, distinctly longer than that for essential blepharospasm or Meige syndrome. Lagophthalmos and exposure keratopathy are also distinctly more common in these patients. Long-term management of these patients has been possible with repeated injections. Other than transient exposure and ptosis, complications are uncommon. Most patients find this approach more acceptable than craniotomy.

Spasmodic Torticollis (Twisted Neck)

Adult-onset spasmodic torticollis[12,34,39,87] represents a form of cervical dystonia characterized by involuntary contractions with an abnormal increase in cervical muscle resting tone (Fig. 6). Although it usually presents as an isolated syndrome, this disorder may develop in patients diagnosed with essential blepharospasm or Meige syndrome. Spasmodic contractions can lead to posture deformity, cervical pain, decreased active range of motion of the head, varying amplitudes of

head tremors, and noticeable hypertrophy within the sternomastoid, splenius capitis and cervicis muscles, levator scapulae, trapezius, and scalene muscles. Routine activities often become difficult, and persisting disfigurement and chronic pain impair the normal lifestyle.

Prior therapy has included the use of neuroleptic medicines and various forms of denervation surgery. Unfortunately, these therapeutic measures often fail to provide a sustained beneficial response. Complications of surgery include lack of efficacy, weakness from paralysis, and disfigurement.

Botulinum A toxin injections have provided a substantial therapeutic resource for this patient population.[12,34,39,87] In preliminary studies, relief of pain has been noted in 82% of patients, as well as improvement of posture deformity (70%), increased range of motion (76%), and reduction in visible hypertrophy (85%).[12] The major complication of therapy has been dysphagia.[11] Retrospective and prospective clinical studies have indicated, however, that dysphagia may be related to dose and the injection strategy used. This complication is significant because dysphagia has been associated in several patients with upper airway obstruction after swallowing large pieces of meat. The incidence of this complication has been reduced by limiting the dose given to the sternocleidomastoid muscle.[11]

Because spasmodic torticollis represents a disease of the coordination centers within the central nervous system, multiple muscles of the neck at remote locations are involved. To achieve a higher degree of efficacy, multiple muscle injections are necessary. Prospective clinical data have recently indicated that the technique of multiple injection points per muscle is superior to a single injection per muscle (Table 1). A subclassification of the various patterns of cervical muscular involvement may prove helpful in formulating an injection strategy for each patient.[12] Four subtypes of spasmodic torticollis have been described based on posture deformity and the pattern of dystonic muscle involvement to facilitate toxin administration.[12]

Effective treatment of this disorder requires higher dose administration than in the management of Meige's disease and blepharospasm and has been associated with the development of immunologic resistance to the toxin. Neutralizing antibodies in these patients appear to render the botulism toxin ineffective.

Other Applications

Other diseases with pathophysiology involving involuntary spasmodic contractions that have been studied under clinical protocols include occupational hand dystonia (writers cramp, musicians dystonia),[31] spasmodic dysphonia,[52] neurogenic bladder from spasmodic contraction of the external urethral sphincter,[28] adductor leg spasms from multiple sclerosis,[86] and jaw dystonia.[4] Efficacy has been demonstrated in each of these conditions. Further clinical studies of long-term management of many of these conditions are in process.

CONCEPT OF DENERVATION FIELD AND INNERVATION ZONES

Because the primary mode of action of botulinum toxin involves partial denervation within a given muscle region and because most complications to date have involved undesirable diffusion of the toxin to contiguous regions, methods for focusing the denervation zone would be clinically helpful. Scott[69]

Table 1. *Response Rates to Botulinum Toxin for Spasmodic Torticollis Comparing Single- and Multiple-Point Injection Strategies*

	Single Point	Multiple Point	Chi-Square
Total dose	161 (average)	151	
Pain	15/31	27/31	$P < 0.002$
Posture deformity	13/42	33/44	$P < 0.001$
Range of motion	15/39	33/44	$P < 0.001$
Activity	13/39	29/38	$P < 0.001$
Muscle hypertrophy	27/39	34/44	$P = 0.330$
Tremor	4/17	9/17	$P = 0.080$

proposed the use of antitoxin to contain "toxin jump." Another approach to this problem is the development of an injection protocol that is based on a defined denervation field produced by a unit quantity of the toxin. The denervation field can be defined by measurement of extrajunctional spread of acetylcholinesterase and careful quantization of muscle fiber size variability.[11] Such future work may be useful in improving the results obtained with this valuable therapeutic agent. Furthermore, a greater understanding of the distribution of anatomic innervation zones (neuromuscular junction maps) within various muscles may be useful in planning therapeutic injections.

CONSISTENCY OF BIOLOGIC ACTIVITY

As just indicated, containment of toxin effect is important for a safe, effective, and consistent clinical response. Recently, because of variations in biologic activity in the product distributed by the current supplier (Allergan), the FDA has not approved several batches of the material for medicinal distribution. Thus, an interruption in drug availability in the United States has occurred. Because "toxin jump" is a major phenomena resulting in complications (e.g., ptosis, dysphagia, diplopia), consistent standardization of biologic activity is critical in the evolution of this technology.

SUMMARY

Botulinum A toxin has been useful alone or as an adjunct for the treatment of strabismus, facial dyskinesia, spasmodic dysphonia, and spasmodic torticollis. Its efficacy seems to be improved by careful anatomic placement of the injections and the use of multiple injection protocols. Side effects are regional and appear to be related to unwanted toxin diffusion. Defined denervation zones as a function of the unit dose of the toxin and further knowledge of innervation zones of muscles may be useful in the design and the development of more efficacious treatment strategies. Consistent standardization in the biologic activity within the vials represents a current manufacturing problem that necessitates further research.

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Address reprint requests to

Gary E. Borodic, MD
100 Charles River Plaza
Boston, MA 02114